Males with prolactinoma are at increased risk of incident cardiovascular disease

Karavitaki, Niki; Toulis, Konstantinos; Kumarendran, Balachandran; Gokhale, Krishna; Cheng, Kar; Nirantharakumar, Krishnarajah

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Complete List of Authors:	Toulis, Konstantinos; University of Birmingham, Institute of Applied Health Research; 424 Army General Training Hospital, Endocrinology Robbins, Tim; University Hospitals Coventry and Warwickshire NHS Trust, Endocrinology Reddy, Narendra; University of Birmingham, Institute of Applied Health Research Kumarendran, Balachadran; University of Birmingham, Institute of Applied Health Research Gokhale, Krishna; University of Birmingham, Institute of Applied Health Research Wijesinghe, Haren; University of Birmingham, Institute of Applied Health Research Cheng, Kar Keung; University of Birmingham, Public Health, Epidemiology, and Biostatistics Karavitaki, Niki ; University of Birmingham, Institute of Metabolism and Systems Research, College of Medical and Dental Sciences; Birmingham Health Partners, Centre for Endocrinology, Diabetes and Metabolism ; Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Endocrinology Wass, John A.H.; University of Oxford, Department of Endocrinology and Diabetes Nirantharakumar, Krishnarajah; University of Birmingham, Institute of Applied Health Research
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Title Page

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Authors: Konstantinos A. Toulis[1,2*], Tim Robbins[3,4*], Narendra Reddy[5], Kumarendran Balachandran[1], Krishna Gokhale[1], Haren Wijesinghe[1], Kar Keung Cheng[1], Niki Karavitaki[6,7#], John Wass[8#], Krishnarajah Nirantharakumar[1,6,7#]

*Joint First Authors # contributed equally

Affiliations:

1 Institute of Applied Health Research, University of Birmingham, B15 2TT, Birmingham, UK

2 Department of Endocrinology, 424 General Military Hospital, Thessaloniki, Greece

3 University Hospitals Coventry and Warwickshire, UK

4 Warwick Medical School, University of Warwick, UK

5 University Hospitals of Leicester, UK

6 Institute of Metabolism and Systems Research, College of Medical and Dental Sciences, University of Birmingham, B15 2TT, Birmingham, UK

7 Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, B15 2TH, UK

8 Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford

Corresponding author:

Dr. Niki Karavitaki, MSc, PhD, FRCP

Institute of Metabolism and Systems Research (IMSR), College of Medical and Dental Sciences, University of Birmingham, IBR Tower, Level 2, Birmingham, B15 2TT, UK

Tel.: 0121 414 3826

Fax: 0121 415 8712 17

E-mail: n.karavitaki@bham.ac.uk

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Abstract

Objective To investigate whether the risk of incident cardiovascular disease (CVD) is increased in patients with prolactinoma.

Design Population-based, retrospective, open-cohort study using The Health Improvement Network (THIN) database.

Patients 2,233 patients with prolactinoma and 10,355 matched controls (1:5 ratio) from UK General Practices contributing to THIN were included. Sex, age, body mass index, and smoking status were used as matching parametres. The primary outcome was any incident CVD, defined by Read codes suggesting myocardial infarction, angina pectoris, stroke, transient ischaemic attack or heart failure. Sex-specific adjusted incidence rate ratios (aIRRs) were calculated with Poisson regression, using clinically relevant parameters as model covariates. Sensitivity analyses were performed to check whether a change in the initial assumptions could have an impact on the findings.

Results During the 6-year observation period, the composite CVD outcome was recorded in 54 patients with prolactinoma and 180 "non-exposed" individuals. The incidence rate was 1.8 and 14.8 per 1000 person-years for the females and males with prolactinoma, respectively. The aIRRs for CVD were estimated at 0.99 [95% Confidence Interval (CI): 0.61-1.61,

p=0.968)] in female patients and 1.94 (95% CI: 1.29-2.91, *p*-=0.001) in male patients. These findings remained robust in sensitivity analyses restricting to patients with documented record of dopamine agonist treatment and those with newly diagnosed prolactinoma.

Conclusions In contrast to females, men with prolactinoma have increased risk for incident CVD; the aetiology of this gender-specific finding remains to be elucidated

Introduction

Prolactinomas are the most common type of pituitary adenoma with prevalence between 34 and 44 cases per 100,000 population [1-5]. Their presenting manifestations relate to the consequences of hyperprolactinaemia (hypogonadism, galactorrhea) and to their potential mass effects (mostly headaches, visual deterioration and pituitary hormone deficits) [6]. The median age at diagnosis is 31-32 years in females and 39-48 years in males, thereby affecting individuals with long life expectancy [1-3]. The documented diagnostic delay reflecting the minimum period to high prolactin (PRL) exposure ranges between 0.5-12 years [1], and macroadenomas, with the potential to cause various degrees of hypopituitarism, account for 19-24% of the total cases and up to 75% of the male patients [1, 2, 5]. First line treatment is dopamine agonists, with cabergoline achieving normal PRL in approximately 90% of microadenomas and 60-90% of macroadenomas. In cases of resistance or intolerance to medical treatment, surgery combined or not with radiotherapy are further options, with various success rates and complications [7-9]

Apart from the impact on the hypothalamo-pituitary-gonadal axis, untreated hyperprolactinaemia has been associated with metabolic derangement and insulin resistance [10-12]. These observations are consistent with the sympatholytic effects on D2-dopamine receptors which are currently studied for the treatment of diabetes mellitus type 2 [13, 14]. It has been also shown that patients with untreated newly diagnosed prolactinoma demonstrate a

hypercoagulable state, reflected in elevated total cholesterol, low density lipoprotein cholesterol, apolipoprotein B, platelet count, fibrinogen, plasminogen activator inhibitor-1 (PAI-1), alongside reduced plasma tissue factor pathway inhibitor levels[15]. However, these reports were universally confirmed in the literature[16].

Adequately powered studies systematically assessing the risk of cardiovascular disease (CVD) in patients with prolactinoma (directly through the hyperprolactinaemia *per se* or indirectly through associated hypopituitarism) are not available. We, thus, for the first time, undertook a population-based, retrospective, open cohort study aiming to clarify the long-term cardiovascular risk in these patients by comparing them to appropriately matched controls.

Materials and Methods

Study design

This was a population-based, retrospective, open cohort study in which patients with the diagnosis of prolactinoma were compared to age, sex, body mass index (BMI) and smoking status matched controls who did not have this diagnosis.

Source of data

Patient data was sourced from The Health Improvement Network database (THIN). THIN data are generated from longitudinal data documented in electronic medical records by General Practitioners during each episode of consultation using Read Codes (a hierarchical coding system for structured storage of information) [17]. More than 675 practices, scattered representatively around the UK, contribute data to THIN covering 3·7 million active patients (6-7% of UK population) [18]. THIN data are generalizable for the UK for major health conditions [19].

Selection of the study population

The study cohort consisted of two sub-cohorts; the "exposed" , including patients diagnosed with prolactinoma and the "non-exposed" one (controls, matched on a 5:1 ratio to each "exposed" subject) with no diagnosis of prolactinoma before or during the observation period. The "exposure" was defined by a Read code specific for prolactinoma (detailed list of Relevant Read Codes are available in the Appendix). Records of any dopamine agonist treatment (cabergoline, bromocriptine, quinagolide) were also collected. Controls were matched to age at index date (to within 1 year), sex, BMI (to within 2 Kg/m2) and smoking status (current smoker or not). These matching variables were selected on the basis of biological plausibility and relevance to CVD. The main outcome was any new (incident) diagnosis of ischaemic heart disease, myocardial infarction, angina pectoris, transient ischaemic attack or stroke or incident diagnosis of heart failure or left ventricular dysfunction (Supplementary Appendix). Cardiac valve disease was not considered in the analysis. Due to power considerations, this was treated as a composite outcome in the analysis. Sex-specific data extraction and analyses were performed.

The THIN data collection scheme received Multi-centre Research Ethics Committee (MREC) approval in September 2003 with Scientific Review Committee (SRC) approval of this study protocol in March 2015 (Ref: SRC13-080).

Observation period

The study period was set from 1st Jan 1990 to 1st September 2015. Each patient diagnosed with a prolactinoma was followed up from their index date (start of observation at the patient level) until the patient died, left the Practice, the Practice ceased data collection or a positive study outcome (cardiovascular event) was recorded. Patients with CVD recorded any time prior to the index date (at baseline) were excluded from the study (only incident CDV was

considered). Observation period and study entry requirements were identical in the control cohort.

Sensitivity and subgroup analyses

Given the observational nature of the evidence, sensitivity analyses were performed aiming to check whether a change in the initial assumptions could have an impact on the findings. Thus, an alternative definition of "exposure", namely a Read code specific for prolactinoma and a concurrent documented treatment with any dopamine agonist, was used in a sensitivity analysis to further consolidate the diagnosis of prolactinoma. Furthermore, a sensitivity analysis was also undertaken limiting to those patients with an incident diagnosis of prolactinoma (patients with a new diagnosis after joining Practice) and their respective controls aiming to diminish the bias associated with the inclusion of prevalent cases. Finally, since prolactinomas are diagnosed at an earlier age in women [1, 2], a subgroup analysis limiting to those female patients aged above 45 years and their respective controls was also undertaken to offset any bias related to the low risk for CVD in premenopausal women.

Statistical analyses

Baseline characteristics (age, follow-up period, sex, Townsend deprivation index [20], BMI, smoking status, presence of hypertension or diabetes mellitus and use of lipid lowering medications) were descriptively analysed. Comparison of baseline characteristics between "exposed" and "non-exposed" groups was performed by appropriate descriptive statistics (Chi-squared, Student's t or Mann-Whitney U tests).

Crude (unadjusted) incidence rate ratios (IRRs) were calculated for each outcome. Adjusted incidence rate ratios (aIRRs) were calculated using Poisson regression model adjusting for patient level covariates. Covariate adjustment analysis was conducted to address the potential impact of imbalance in baseline characteristics. Covariates were age, sex, categories of BMI (<25, 25-29.9, ≥30 Kg/m2 and missing values groups), deprivation quintiles,

hypertension, diabetes mellitus, use of lipid lowering medications and smoking status. IRRs were calculated with 95% confidence intervals (CI) and a statistical significance threshold taken to be $p<0.05$. Applying multiple significance tests was avoided to minimise inflation of alpha error [21] and as per recommendation of the RECORD guideline for reporting epidemiological studies using routinely collected data [22]. All statistical analyses were performed using Stata 14.0 software (StataCorp. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP)

Results

Baseline characteristics

A total of 2,233 prevalent (diagnosed before the index date) and incident (diagnosed after the index date) patients with prolactinoma (1,822 females and 411 males) and no history of CVD at baseline were identified. After the identification of the "exposed" patients, out of the pool of individuals with no prolactinoma, a total of 10,355 subjects (8,557 females and 1,798 males) were randomly selected on 1:5 ratio, matching on sex, age, BMI and smoking status. The study population consisted of a total of 12,588 individuals (10,379 females and 2,209 males) with mean age 37.1 (SD 10.2) and 47.3 (SD 14.4) years for females and males, respectively. The baseline characteristics of the subjects of the study are shown in Table 1. There was no significant difference in age, smoking status, presence of hypertension or use of lipid lowering medications between the "exposed" and "non-exposed" cohort at baseline. Although BMI was matched to within 2 Kg/m2 between the "exposed" and "non-exposed" individuals, this was marginally but statistically different between the two groups for both males and females as a result of the large sample size. Diabetes mellitus was significantly more frequent in the "non-exposed" subjects. The potential impact of these imbalances was further addressed by covariate adjustment analysis.

Main Outcome

During the observation period, the composite CVD outcome was recorded in 54 (20 females and 35 males) patients with prolactinoma and 190 (103 females and 87 males) "non-exposed" individuals. The incidence rate for the "exposed" females was 1.8 per 1000 person-years compared to 2.0 per 1000 person-years for the "non-exposed" females. The incidence rate for the "exposed" males was 14.8 per 1000 person-years compared to 8.7 per 1000 person-years for the non-exposed" males.

The crude (unadjusted) IRR for CVD in female patients compared to matched controls was estimated at 0.90 [95% CI: 0.56-1.45, p=0.666]. After adjusting for age, gender, deprivation quintiles, BMI groups, hypertension, smoking, lipid lowering medications and diabetes mellitus, the aIRR was found to be similar and was estimated at 0.99 (95% CI: 0.61-1.61, p=0.968).

The crude IRR for CVD in male patients with prolactinoma was found to be significantly higher compared to matched controls and was estimated at 1.72 (95% CI: 1.16-2.55, p=0.001). After covariate adjustment, the aIRR changed minimally and was estimated at 1.94 (95% CI: 1.29-2.91, p=0.001). The findings of the above analyses are presented in detail in Supplementary Appendix.

Sensitivity and subgroup analyses

Excluding patients with no record of dopamine agonist treatment and their respective controls did not alter the main findings: aIRR was calculated at 1.13 (95% CI: 0.61-2.09, p=0.689) for female and 1.98 (95% CI: 1.27-3.09, p=0.002) for male patients. A detailed presentation of this analysis is shown in Table 2. Sensitivity analysis limiting to incident cases and their respective controls revealed similar findings: aIRR was estimated at 1.04 (95% CI: 0.54-2.03, p=0.894) for female patients and 2.00 (95% CI: 1.14-3.49, p=0.019) for male patients. A detailed presentation of this analysis is shown in Table 2. Sensitivity analysis treating each component of the composite cardiovascular outcome as a separate outcome (namely

ischaemic heart disease, stroke/TIA, heart failure/left ventricular dysfunction) revealed that the results were consistent in both male and female patients. Similarly, the exclusion of two patients with concurrent acromegaly did not alter the findings. Routine surveillance for cardiac valve disease in some patients with prolactinoma may have resulted in high detection of left ventricular dysfunction. However, excluding heart failure from our composite outcome did not alter our findings. Finally, when analysis was restricted to those female patients diagnosed with prolactinoma who are above 45 years and their respective controls, the IRR was at 1.02 (95% CI: 0.54 – 1.90, p=0.95).

Discussion

This is the first population-based, retrospective, open cohort study looking systematically at the cardiovascular morbidity in patients with prolactinoma. We have shown that males have a higher incidence of CVD compared to matched subjects without this diagnosis over a six year observation period (IRR 1.72 (95% CI: 1.16–2.55, p=0.001)]. In contrast, there is no evidence to suggest an increase in the risk of CVD in female patients with prolactinoma. These findings were also confirmed after adjustment for clinically significant covariates and remained robust in sensitivity analyses.

Studies systematically assessing the risk of CVD in adequately powered sample of patients with prolactinoma are not available. Possible mechanisms affecting the cardiovascular morbidity in this group of patients include a direct effect of hyperprolactinaemia, as well as the impact of potential pituitary hormone deficits and/or their management.

In population-based studies, it has been previously shown that the levels of PRL associate positively with inflammatory biomarkers (such as interleukin-6) [23], adverse cardiovascular risk profile [15] and increased cardiovascular mortality [24]. Furthermore, particularly in patients with untreated prolactinoma, a range of metabolic disorders (including insulin resistance,

elevated total cholesterol, low density lipoprotein cholesterol, apolipoprotein B), deranged fibrinolysis (platelet count, fibrinogen, PAI-1 and PAI-1/ tissue plasminogen activator ratios), as well as evidence of preclinical atherosclerosis have been reported [10-12, 25-28]. Although the duration of hyperprolactinaemia is not known in our cohort of prolactinoma patients, published literature suggests diagnostic delays ranging between 0.5-12 years reflecting the minimum period of exposure to high PRL[1]. Whether the impact of previous hyperprolactinaemia on the cardiovascular system is reversible or persists despite treatment with dopamine agonists remains to be elucidated.

Interestingly, we found that the increased risk for CVD in male patients persisted even in the presence of concurrent documented treatment with dopamine agonist; the inclusion of cases with suboptimal biochemical control (due to resistance, intolerance or non-compliance) cannot be excluded, particularly given that male gender has been independently associated with resistance to cabergoline [29]. It should be also noted that the duration of exposure to high PRL levels may be a significant effect modifier, which is particularly relevant when investigating outcomes like CVD and may provide a possible explanation for the gender differences we identified. In line with this, males are diagnosed at an older age than females, possibly implying longer diagnostic delay and exposure to the consequences of hyperprolactinaemia and of related hypogonadism[1]. "Interestingly, a recent retrospective cohort study including approximately 373 individuals with hyperprolactinemia (irrespective of its primary aetiology) reported similar findings with our study [30]. In this report, male hyperprolactinaemic patients had a higher IRR for cardiovascular and all-cause mortality in contrast to female patients, in whom no difference was noted when compared to normoprolactinaemic controls [30]. Of note, an older study of a case-control design which explored prolactin levels in those who suffered a coronary artery event and controls did not find higher prolactin levels in the affected patients [31]. This was the case (non-significant

findings) in another study of a cohort design, however the hyperprolactinaemic patients were few [32] and possibly the study was underpowered.

Hypopituitarism is associated with increased cardiovascular morbidity [33] and is diagnosed in patients with adenomas large enough to cause damage to the normal adenohypophyseal cells. A limitation of the present study was the inability to discriminate between micro- or macroprolactinomas. However, given that macroprolactinomas are more common in males [1], the possibility that men with prolactinoma are most likely to have hypopituitarism, cannot be excluded; this hypothesis can provide a further explanation on our gender-specific findings. In this line of thought, it would be clinically relevant to include a control group with patients diagnosed with non-functioning pituitary adenoma. Unfortunately, this was not currently feasible in the THIN database.

Analysis restricted to those female patients who are aged above 45 years and their respective controls still did not confirm high IRR for CVD [1.02 (95% CI: 0.54 – 1.90, p=0.95)]. Whether a longer duration of follow-up would alter these results needs to be clarified.

The advantages of our study are that it is population-based with large sample size and appropriate matching for confounding factors. Furthermore, we performed sensitivity analyses, which enhanced the validity of the original results. Limitations include the lack of detailed clinical phenotyping (adenoma size, pituitary dysfunction and its management, response to dopamine agonist treatment, other treatments used for the prolactinoma), which would allow further clarification of the pathogenetic mechanisms of our findings. Moreover, it should be noted that patients with a documented history of CVD event preceding the index date were excluded from the study to ensure outcomes could be attributable to the diagnosis of prolactinoma and not to other pre-existing risk factors of CVD. This may have resulted in a population at low risk for CVD, which may not be reflective of the general population of patients with prolactinoma. Finally, the validity of prolactinoma-related recordings is not

fully documented in THIN as yet. Nonetheless, large well-characterised patient registries may facilitate this in the future and will also allow causal interpretation of our observational data.

In conclusion, in a population-based, retrospective cohort study of 12,588 subjects, we have found that incident CVD is increased only in men with prolactinoma. Long-standing hyperprolactinaemia and its consequences, as well as hypopituitarism and its management may be the underlying mechanisms. The impact of these findings on the long-term mortality of these patients remains to be reviewed.

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Tables

Table 1: Baseline characteristics of study population

	Females (n=10,379)		Males (n=2,209)	
	Prolactinoma subjects	**"Non-exposed" subjects**	**Prolactinoma subjects**	**"Non-exposed" subjects**
Number of subjects	1,822	8,557	411	1,798
Follow-up period (years)*	6.1 [5.2]	6.0 [4.9]	5.6 [4.7]	5.6 [4.6]
Age (years)*	37.1 (10.2)	37.1 (10.2)	47.2 (14.5)	47.4 (14.4)
Body mass index*	26.7 (6.3)	26.0 (5.4)*	29.6 (6.1)	28.1 (4.6)*
Current smoking	276 (15.2)	1,237 (14.5)	63 (15.3)	267 (14.85)
Hypertension	95 (5.2)	510 (6.0)	65 (15.8)	333 (18.5)
Lipid lowering medications	53 (2.9)	278 (3.3)	55 (13.4)	264 (14.7)
Diabetes mellitus	24 (1.3)	217 (2.5)*	19 (4.6)	148 (8.2)*
Townsend index				
(Least deprived) 1	416 (22.8)	1,932 (22.6)*	111 (27.0)	447 (24.9)
2	324 (17.8)	1,711 (20.0)	90 (21.9)	416 (23.1)
3	414 (22.7)	1,749 (20.4)	90 (21.9)	356 (19.8)
4	348 (19.1)	1,643 (19.2)	52 (12.7)	305 (16.9)
5	189 (10.4)	1,008 (11.8)	38 (9.2)	186 (10.3)
Not available	131 (7.2)	514 (6.0)	30 (7.3)	88 (4.9)

Results for continuous variables are presented as mean (standard deviation) and for dichotomous and ordinal variables as N (%). A high Townsend index is indicative of high material deprivation. The index is assigned to each patient record based on their residential postcode. For diabetes mellitus, hypertension and smoking status, a positive documentation in the General Practice records was considered as presence of the risk factor. * Statistically significant at 0.05

Table 2: Sensitivity analyses restricting to those with evidence of treatment with dopamine agonist or incident diagnosis of prolactinoma and their respective controls

	Female patients with evidence of dopamine agonist therapy		Male patients with evidence of dopamine agonist therapy		Incident female patients		Incident male patients	
	Prolactinoma subjects	**"Non-exposed" cohort**	**Prolactinoma subjects**	**"Non-exposed cohort"**	**Prolactinoma subjects**	**"Non-exposed" cohort**	**Prolactinoma subjects**	**"Non-exposed" cohort**
Number of subjects	1,312	6,147	353	1,546	795	3,718	232	1,025
Person-years	8,331	37,092	2,006	8,732	5,291	23,138	1,259	5,421
Incident cardiovascular disease	13	53	29	74	11	50	18	52
Incidence Rate (per 1000 person-years)	1.6	1.4	14.5	8.5	2.1	2.2	14.3	9.6
IRR (95% CI)	1.09 (0.60-2.00)		1.70 (1.11-2.62)		0.96 (0.50-1.85)		1.49 (0.87-2.55)	
p	0.776		0.015		0.907		0.145	
Adjusted IRR (95% CI)*	1.13 (0.61-2.09)		**1.98 (1.27-3.09)**		1.04 (0.54-2.03)		**2.00 (1.14-3.49)**	
p	0.689		0.002		0.894		0.019	

*Adjusted for age, gender, deprivation quintiles, body mass index (BMI) group, hypertension, smoking, lipid lowering medications and diabetes mellitus. The BMI categories (kg/m^2) were <25, 25-29.9, ≥30 and missing values groups. *P* - values were derived from Poisson regression. CI: Confidence Interval, IRR: Incidence Rate Ratio

Supplementary Appendix

Contents

- Further methodological details on the construction of the cohort
- Summary of the Read codes used for the study
- Supplementary Table: Risk of incident cardiovascular disease on the basis of prolactinoma diagnosis

Further methodological details on the construction of the cohort

- The index date for each exposed patient (start date of patient-specific observation) was set at one year after registration with the Practice (if the patient was already diagnosed with prolactinoma and the Practice was eligible for inclusion in THIN) or the date the Practice became eligible for participation (if the patient was already diagnosed with prolactinoma and the Practice initially not eligible) or the date of the first diagnosis (incident patients with prolactinoma), whichever was the latest.
- Individual Practices (already included into the THIN network) were eligible for inclusion in the study from the later of the following two dates: one year after the date their Practice system was installed; and the Practice's acceptable mortality recording (AMR) date (a measure of quality of the data). This approach ensured that any selected Practice was making full use of their system and was not under-recording important outcomes. This minimum entry period of one year from the patient's Practice registration date was applied to maximise the likelihood that each case had sufficient time to have their baseline characteristics and comorbidities recorded in the system.
- A minimum one year entry period after registration with the Practice described above was applied in the controls ("non-exposed patients) as well. Individuals were followed up until death, departure from Practice, cessation of Practice data collection or positive outcome (incident cardiovascular event). Similarly, any control patient who had the outcome of interest preceding the index date was also excluded from the study.

Summary of the Read codes used for the study

Exposure: BB5y400 (prolactinoma)

Outcomes:

Cardiovascular Disease

Ischaemic Heart Disease mainly driven by: G3... (IHD), G30... (Myocardial Infarction) G33..... (Angina Pectoris)

Stroke and TIA mainly driven by : Codes stemming from G6... (Cerebrovascular Disease)....G65 (TIA).....G66 (stroke)

Observed Read Codes for outcomes:

IHD, stroke, TIA	Count of PRACTICE_PATIENT ID
G3...00	7
G3...13	3
G30..00	3
G30..15	1
G307100	1
G30X000	1
G311100	1
G311500	2
G33..00	9
G340.11	1
G340.12	2
G340100	1
G6...00	1
G60..00	1
G61..00	1
G623.00	1
G64..00	1
G64..11	2
G64..13	1
G640000	1
G64z.00	1
G64z200	1
G64z400	1
G65..00	5
G65..12	4
G65zz00	1
G66..11	1
G66..12	1
G667.00	1
G6X..00	1
Total	58

Supplementary Table: Risk of incident cardiovascular disease on the basis of prolactinoma diagnosis

	Females		**Males**	
	Prolactinoma	**"Non-exposed" cohort**	**Prolactinoma**	**"Non-exposed" cohort**
Number of patients	1,822	8,557	411	1,798
Incident cardiovascular disease	20	103	34	87
Person-years	11,112	51,489	2,287	10,052
Incidence Rate (per 1000 person-years)	1.8	2.0	14.8	8.7
Incidence Rate Ratio (95% CI)	0.90 (0.56-1.45)		1.72 (1.16-2.55)	
p	0.666		0.007	
Adjusted Incidence Rate Ratio (95% CI)	0.99 (0.61-1.61)		**1.94 (1.29-2.91)**	
p-	0.968		0.001	

Adjusted for age, gender, deprivation quintiles, body mass index (BMI) group, hypertension, smoking, lipid lowering medications and diabetes mellitus. The BMI categories (kg/m^2) were <25, 25-29.9, >=30 and missing values groups. *P*-values were derived from Poisson regression.